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Evaluation of Klebsiella infections in the tertiary neonatal intensive care unit

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Abstract

Aim: In this study, we aimed to determine the frequency, demographic characteristics, associated risk factors, resistance patterns and factors affecting mortality and morbidity of Klebsiella infections in hospitalized neonatal patients.

Material and Methods: Neonates who were identified with culture-proven Klebsiella infection in NICU were included in the patient group, and those who were admitted to the same unit on the same day and did not suffer from Klebsiella infection were selected as a control group.

Results: One hundred nine patients and 417 patients were included in the study as patient group, and control group, respectively. Extended-spectrum β-lactamase (ESBL) producing K. pneumoniae was detected in 79% of the patients while CRKP infection was detected in 26%. Fetal distress exposure (p=0.032), prematurity (p=0.004), prior hospitalization (p=0.024), peripherally inserted central venous catheterization (p=0.018), urinary (p=0.003) and nasogastric catheterization (p=0.003), total parenteral nutrition (TPN) use (p=0.008), and long-term hospitalization (p<0.001) were found to be risk factors for Klebsiella infection. The sensitivity of colistin and meropenem were 100% and 87.9% in antibiograms. Previous antibiotic use (p=0.002) and mortality (p=0.033) were higher in patients with CRKP infection compared to the carbapenem sensitive patient group. CRKP infection developed in 21% of patients with CRKP colonization.

Discussion: Prior hospitalization, prematurity, and invasive procedures are important risk factors for Klebsiella infections in neonates. Mortality and previous antibiotic use are much higher in patients with CRKP infection than in the carbapenem sensitive group. To prevent and control Klebsiella infections, minimally invasive procedures, strict infection control protocols, and rational use of antibiotics are required. Overuse of colistin should be limited to prevent colistin-resistant Klebsiella outbreaks in NICUS in the near future.

Keywords

Carbapenems, Carbapenem-Resistant Enterobacteriaceae, Colistin, Klebsiella, Neonate, Neonatal Intensive Care Unit

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Introduction

Klebsiella infections cause epidemics in neonatal intensive care units (NICU), resulting in severe morbidity and mortality and high healthcare costs [1, 2]. Infections caused by carbapenem-resistant *Klebsiella pneumoniae* (CRKP) have increased over the past two decades [3], and mortality rates range from 33 to 50% [3]. Therefore, the rapid and global spread of Klebsiella infections raises concerns in healthcare settings. In order to prevent and control these infections and to evaluate the antibiotic beginning regimens, it is essential to reveal the clinical and epidemiological features of the infections, as well as the risk factors.

The purpose of this study was to determine the frequency, epidemiological characteristics, associated risk factors, resistance patterns, and factors affecting mortality and morbidity of infections with *K. pneumoniae* in hospitalized neonatal patients.

Material and Methods

Study group

This descriptive study was conducted at the Neonatal Clinic of Dr. Sami Ulus Obstetrics and Gynecology and Pediatrics Training and Research Hospital, and was approved by the local Ethics Committee of the same center in 2017, March (E-73799008-799). Patients who were hospitalized in NICU and had culture-proven Klebsiella infection were included in the study group. For each patient, 4 neonates who were hospitalized in the same service on the same day and did not suffer from Klebsiella infection were selected as a control group. The clinical and demographic data of the patients were reviewed from electronic records retrospectively. Klebsiella growth in anal swab culture was detected in 28 neonates. Among these, neonates who were not diagnosed with clinical sepsis (n=16) were considered colonized, and were excluded from the study group. Among sepsis patients, cases with a postnatal age of less than 72 hours were classified as early onset sepsis (EOS), and those ≥72 hours as late-onset neonatal sepsis (LOS). Infections such as bloodstream infection (BSI), catheter-related BSI, ventilator-associated pneumonia (VAP), urinary tract infection, peritonitis, omphalitis, meningitis, skin abscess and osteomyelitis acquired at least 48 hours after hospitalization and within 10 days of discharge were defined as healthcare-associated infections (HAI) [4, 5].

Laboratory evaluation

Blood samples of the patients were inoculated into BacT/ALERT PF Plus blood culture bottles [bioMerieux, France] and incubated in the BacT/ALERT system (bioMerieux, France) for 5 days. Upon receiving a positive signal from the device, blood culture bottles were transferred to 5% sheep blood agar, chocolate agar, and EMB (Eosin Methylene Blue) agar. Plates were incubated at 35ºC with 5% CO2. After evaluating the colony morphology and Gram staining of the growing microorganisms, manual methods or GN colorimetric identification cards of the VITEK 2 Compact automated system (bioMerieux, France) were used for species-level identification. The oxidase test, lactase fermentation test, indole test, methyl red test, motion test, citrate test, three sugar fermentation, and urease test were used as manual procedures.

Manual antibiotic susceptibility methods or GN AST cards of the VITEK 2 Compact automated system (bioMerieux, France) were used to determine the antibiotic susceptibility of the isolates. The Kirby-Bauer disc diffusion and E-test methods were used as a manual antibiotic susceptibility test, and the results were evaluated according to CLSI [72] and EUCAST [72] criteria, respectively. The Double Disc Induction Method was used to detect inducible beta-lactamase (IBL), whereas the Double Disc Synergy Test was used to detect extended-spectrum beta-lactamase (ESBL). Gradient strip test was used to re-evaluate the carbapenem-resistant isolates that were determined to be resistant using human or automated methods. Leukocyte and neutrophil values were evaluated according to Nathan and Oski's book on Pediatric Blood Diseases. [6].

Statistical analysis

The analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS) statistical package program (Version 22.0; SPSS, Inc., Chicago, IL, USA). Chi-square and Student's T tests were used to compare parametric values. Fisher's exact test was used if at least one expected count was less than 5. The level of statistical significance was set at $p < 0.05$.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

The study group consisted of 109 patients infected with Klebsiella, and the control group consisted of 417 patients. K. pneumonia was isolated from 106 (97%) patients, while K. oxytoca was isolated from 3 (3%).

Seventy patients (64%) were male, 66 (61%) were delivered by cesarean section (C/S), and 47 (43%) were preterm. Prematurity was significantly higher in the patient group (p=0.004). The mean birth weight was also significantly lower compared to the control group (2670.55 ± 87.88 gr vs. 2925.87 ± 37.55 gr, p=0.008). Fetal distress was detected in 19 (17%) patients, and was also significantly higher in the patient group (p=0.032).

Fifty patients (46%) were diagnosed with another neonatal infection before Klebsiella infection (urinary tract infection, acute gastroenteritis, pneumonia, omphalitis, sepsis, meningitis). Moreover, 16 patients (15%) were diagnosed with congenital heart diseases, 15 patients (14%) with other congenital malformations, 3 patients (3%) with metabolic diseases, 6 patients (6%) with neurological diseases, and 1 patient (1%) with hematological disease.

EOS was diagnosed in 6 patients (6%) with Klebsiella infection, while LOS was diagnosed in 103 patients (94%). Seventy-five (69%) of these infections were HAI. Of these, 43 were urinary tract infections, 19 were BSIs, 7 were catheter-related BSIs, 5 were VAPs, one was skin abscess. Seven (9%) of those with HAI were from an external center, while 68 (91%) HAIs occurred in our center. All HAIs were LOS and developed on average 31.02 ± 4.9 days after hospitalization.

At the time of the Klebsiella infection, abnormal leukocyte counts (leukopenia and/leukocytosis), thrombocytopenia, and C reactive protein (CRP) levels were significantly higher while hemoglobin levels were significantly lower than in the control group (Table 1).

Table 1. Comparison of demographic, clinical and laboratory features of the patients with K. pneumoniae infection and control group.

	Patients with *K. pneumoniae* infection N=109 (26%)	Control group N=417 (%)	P value
Male gender; no. (%)	70 (64%)	232 (56%)	0.107
Age at hospitalization, days†	8.06 ± 1.53	9.04 ± 1.61	0.824
Cesarean section; no. (%)	66 (61)	222 (53)	0.172
Birth weight, gr†	2670.55±87.88	2925.87±37.55	0.008
Prematurity; no. (%)	47 (43)	120 (29)	0.004
5 minutes Apgar score <7; no. (%)	11 (10)	22 (5)	0.093
Fetal distress; no. (%)	19 (17)	40 (10)	0.032
Prior hospitalization; no. (%)	47 (43)	132 (32)	0.024
Early onset sepsis; no. (%)	6 (5)		
Late onset sepsis; no. (%)	103 (95)		
Healthcare-associated infections; no. (%)	75 (69)		
Invasive procedures; no. (%)			
Operation	20 (18)	52 (13)	0.152
Central venous catheter	18 (17)	48 (12)	0.214
Peripherally inserted central catheter	8 (7)	10 (2)	0.018
Intubation	40 (37)	115 (28)	0.063
Umbilical venous catheter	34 (31)	98 (24)	0.099
Nasogastric tube	55 (51)	146 (35)	0.003
Urinary catheter	25 (23)	47 (11)	0.003
Total parenteral nutrition	49 (45)	131 (31)	0.008
Blood exchange transfusion	1 (1)	9 (2)	0.352
Previous antibiotic use; no. (%)	69 (63)	288 (69)	0.251
Vancomycin	20 (18)	90 (22)	0.544
Meropenem	22 (20)	91 (22)	0.810
Laboratory results			
Leukopenia/leukocytosis; no. (%)	19 (17)	38 (9)	0.005
Thrombocytopenia; no. (%)	24 (22)	18 (4)	< 0.001
Hemoglobin, g/dl†	12.23 ± 0,35	15.49 ± 0.13	< 0.001
CRP elevation (>3.14 mg/dl); no. (%)	57 (52)	115 (28)	< 0.001
Length of hospitalization, days†	44.14 ± 6.25	20.69 ± 1.66	< 0.001
Exitus	17 (16)	35 (8)	0.026

†mean, CRP:C reactive protein

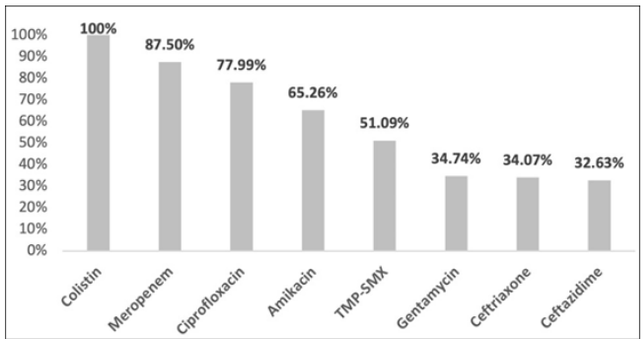

Figure 1. Antibiotic susceptibility patterns of neonatal Klebsiella infections.

Table 2. Comparison of previous antibiotic use and prognosis of the patients with Carbapenem-resistant and Carbapenem-sensitive Klebsiella infections.

	Patients with Klebsiella infection (n=109)		P value
	Carbapenem resistance n=28 (26%)	Carbapenem sensitivity n=81 (74%)	
Previous antibiotic use; no. (%)	25 (89)	44 (54)	0.002
Vancomycin; no. (%)	4 (14)	16 (20)	0.718
Carbapenems; no. (%)	8 (29)	14 (17)	0.313
Exitus, no. (%)	8 (29)	9 (11)	0.033

CRKP infection was detected in 28 (26%) patients. Among these, 6 (21%) patients had CRKP colonization before.

Sensitivity to colistin was 100.0%, and meropenem was 87.5%, while the sensitivity to ciprofloxacin was 77.9%. Ceftazidime (32.6%), ceftriaxone (34.1%), and gentamicin (34.7%) had the lowest levels of sensitivity (Figure 1).

There were 47 patients with a prior hospitalization history, which was significantly higher than in the control group (43% vs. 32%; p=0.024). The use of peripherally inserted central venous catheters, nasogastric tubes, urine catheters and TPN feeding was significantly higher than in the control group (Table 1). Sixty-nine patients (63%) had been given antibiotics prior to Klebsiella infection. Among these, 20 patients (18%) received vancomycin, and 22 (20%) received meropenem. When antibiotic use before Klebsiella infection was compared, there was no significant difference between the patient and control groups (p=0.251). However, previous antibiotic use was higher in patients with CRKP infection than in the carbapenem sensitive group (89% vs. 52%, p=0.002) (Table 2). Five patients with CRKP infection continued to develop CRKP growths under the treatment of colistin, and three of these patients died.

The patients were hospitalized at postnatal 8.06 ± 1.53 days. The mean length of hospital stay in the patient group was significantly higher than in the control group (44.14 ± 6.25 days vs. 20.69 ± 1.66 days, p<0.001).

In the patient group, 17 (16%) patients died, and mortality was significantly higher compared to the control group (16% vs. 8%, p=0.026). Moreover, among these patients, all had HAI, three were followed up with complex congenital heart diseases, one patient with chronic renal failure, two patients with metabolic disease, two patients with undiagnosed syndromic diseases, one patient with gastroschisis, and 8 patients with prematurity. Eight patients with CRKP infection (29%) died. Mortality in patients infected with CRKP was significantly higher than in patients infected with carbapenem-sensitive Klebsiella (29% vs 11%, p=0.033) (Table 2).

Discussion

Klebsiella is an important cause of death and morbidity leading to HAI despite new discoveries in antimicrobial therapy and advances in supportive therapy, especially in preterm and neonates with low birth weight (LBW).

In this study, 48 patients (44%) were preterm, and the rate of prematurity was significantly higher than in the control group.

Due to an increase in survival rates, prolonged hospitalizations, and exposure to invasive procedures, the incidence of infections in LBW and/or preterm infants increases in the current period. It has been reported that the risk of infection in these neonates is three to ten times higher than in terms with normal birth weight [7].

In this study, among CRKP colonized patients, 6 (21%) developed CRKP infection. Wang et al. showed that 24.5% of neonates with rectal Carbapenem-resistant Enterobacteriaceae (CRE) colonization developed CRE infection [8]. Similarly, Akturk et al. reported that 24 (28.2%) of 85 patients with CRKP colonization in pediatric intensive care and NICUs developed CRKP infection [9]. K. pneumoniae is the predominant microorganism colonizing the gut in neonates with a longer stay in the NICU and in those with prolonged feeding through an enteral tube [10]. Because of the immature immune system, lower levels of mucus and gastric acid production, bacteria are able to penetrate intestinal barrier and cause sepsis. When Klebsiella infection is detected in NICUs, it is imperative to immediately include a swab culture for Klebsiella from all infants hospitalized in the unit in the general precautionary packages to detect colonization early, followed by close monitoring of colonized patients for signs of infection.

In this study, we observed LOS in 95% of the patients. HAIs developed on average 31.02±4.91 days after hospitalization. Fifty patients (46%) underwent three or more invasive operations, and peripherally inserted central venous catheterization (7%), nasogastric tube (51%), urine catheterization (23%), and TPN feeding (45%) were significantly higher in the patient group. Similarly, previous studies have reported that underlying chronic medical conditions, invasive medical devices, and frequent/prolonged hospitalizations are risk factors for CRE infections [3, 11, 12]. We conclude that invasive procedures and prolonged hospitalization increase as patients' gestational weeks decrease, resulting in a decline in barrier functions and providing an entry for infections.

The widespread use of antibiotics in NICUs leads to the spread of multi-antibiotic-resistant bacteria [13]. In this study, Klebsiella showed the most sensitivity to colistin (100%), followed by meropenem (87.5%) and ciprofloxacin (77.9%). The sensitivity to ceftazidime (32.6%), ceftriaxone (%34.1) and gentamicin (34.7%) was the lowest. According to a previous study performed in our unit, Klebsiella strains were 97% sensitive to meropenem, 91% sensitive to ciprofloxacin, 91% to ceftriaxone, and 85% to ceftazidime [14]. In a recent study, all neonates with CRKP were reported resistant to ciprofloxacin, ceftriaxone, and ceftazidime but susceptible to colistin [15]. It is concerning that resistance to meropenem and third-generation cephalosporins has increased dramatically over time. Moreover, although colistin sensitivity was 100%, five patients with CRKP infection developed recurrent CRKP growths under colistin, and three of these patients died. We conclude that in vivo sensitivity of colistin might be low. There are also several studies that reported Colistin-resistant Klebsiella spp. in the last years in NICUs [16, 17]. Due to overuse of this antibiotic, we may face more cases of colistin resistant Klebsiella outbreaks in NICUS in the near future, and treatment options may be limited if colistin resistance develops. Therefore, it is crucial to perform susceptibility testing using phenotypic and genetic/molecular methods in order to prompt timely infection control procedures, and guide clinicians in choosing the most appropriate therapy [18].

In this study, 28 patients (26%) were infected with CRKP. Among these, six patients (29%) died, and all had HAI. In previous studies, the mortality rate of CRKP in NICU ranged from %33 to %40 [19-21]. Consistent with these reports, we showed that the carbapenem-resistant group had a higher mortality rate than the carbapenem-sensitive group. CRE infections (particularly Klebsiella) are considered to be on the rise globally and represent a public health threat [22].

The limitation of our study is that comorbidities predisposing to infection could not be evaluated due to the retrospective nature. In addition, the risk factors associated with CRKP infection could not be evaluated because the number of CRKP infections was insufficient. The advantages of our study include the sample size of 109 patients with Klebsiella infection, which is larger than in other studies, and the presence of a control group.

Conclusion

Klebsiella infections in NICUs are associated with prematurity, prolonged hospitalization, fetal distress, and invasive interventions. Moreover, hospital stay and mortality in neonates with CRKP infection are much higher than in carbapenem sensitive group. Early detection of colonization, prevention of nosocomial transmission, and early tailored diagnostic and therapeutic strategies are required for the management of Klebsiella infections.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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